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● *Original Contribution*

IMAGING OF THE OVINE CORPUS LUTEUM MICROCIRCULATION WITH CONTRAST ULTRASOUND

VASSILIS SBOROS,* M. AVERKIOU,† M. LAMPASKIS,† D. H. THOMAS,* N. SILVA,* C. STROUTHOS,† J. DOCHERTY,‡ and A. S. MCNEILLY‡

*Medical Physics and Centre for Cardiovascular Sciences, Queens Medical Research Institute, University of Edinburgh, Edinburgh, UK; †Department of Mechanical and Manufacturing Engineering, University of Cyprus, Nicosia, Cyprus; and ‡Medical Research Council Human Reproductive Sciences Unit, Queens Medical Research Institute, University of Edinburgh, Edinburgh, UK

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Abstract—Ultrasound contrast agents have been the subject of microvascular imaging research. The sheep corpus luteum (CL) is a microvascular tissue that provides a natural angiogenic and antiangiogenic process, which changes during the luteal phase of the estrous cycle of the ewe. It can also be controlled and monitored endocrinologically, providing a very attractive *in vivo* model for the study and development of microvascular measurement. The perfusion of the fully developed CL between days 8 and 12 of the estrous cycle was studied in six ewes. A Philips iU22 ultrasound scanner (Bothell, WA, USA) with the linear array probe L9-3 was used to capture contrast-enhanced images after an intravenous bolus injection of 2.4 mL SonoVue (Bracco S.P.A., Milan, Italy). Time-intensity curves of a region of interest inside the CL were formed from linearized image data. A lagged-normal model to simulate the compartmental kinetics of the microvascular flow was used to fit the data, and the wash-in time was measured. Good contrast enhancement was observed in the CLs of all animals and the wash-in time averaged at 5.5 s with 9% uncertainty. The regression coefficient was highly significant for all fits. These data correlated with stained endothelial area in the histology performed postmortem. Two ewes were injected with prostaglandin F2alpha to induce CL regression, which resulted in an increase of wash-in time after a few hours. The CL of the ewe is thus proposed as an ideal model for the study and development of microvascular measurements using contrast ultrasound. Our initial results demonstrate a highly reproducible model for the study of the microvascular hemodynamics in a range of tissues and organs. (E-mail: Vassilis.Sboros@ed.ac.uk) © **2011 World Federation for Ultrasound in Medicine & Biology.**

Key Words: **Sheep ovary, Microbubble, SonoVue, Ultrasound, Tracer kinetics, Contrast imaging.**

INTRODUCTION

Ultrasound imaging is a widely used diagnostic tool and, with the introduction of microbubble (MB) contrast agents, it provides an enhanced capability to visualize the vascular space. Imaging of microvascular flow and perfusion have thus been the subject of ultrasound contrast techniques for nearly 20 years (Sboros and Tang 2010), because they would be of diagnostic value to a wide range of diseases. Today, a number of techniques that look into the microvascular space are slowly being adopted in the clinic, mainly in cardiology (Kaufmann et al. 2007) and liver radiology (Quaia 2007; Averkiou et al. 2010). However, a number of problems remain unresolved and an objective quantification method is yet to be established. In cardiology, for example, apical sections of the myocardium compare better than other sections with single photon emission computed tomography as the gold standard (Gudmundsson et al. 2010), which is perhaps related to the unresolved ventricular nonlinear attenuation (Tang and Eckersley 2006). Both the myocardium and the liver, as well as the large number of other organs that have been investigated, offer complex vascularities and challenges for contrast-enhanced ultrasound (CEUS). Current transducer and signal processing technologies have offered excellent tissue cancellation and today "contrast-only" images are commonplace.

Ultrasound contrast MBs remain in the vascular space (Jayaweera et al. 1994; Lindner et al. 2002), unlike other

Address correspondence to: Vassilis Sboros, Medical Physics, School of Clinical Sciences and Community Health, The University of Edinburgh, The Chancellor's Building, 49 Little France Crescent, Edinburgh, EH16 4SB, UK. E-mail: Vassilis.Sboros@ed.ac.uk

imaging modalities' contrast materials. Although MB kinetics cannot thus offer a true representation of perfusion, which incorporates permeability and nutrient delivery, it offers the advantage of simplicity. With the available evidence, the measurement of microvascular or capillary blood flow and volume should be attainable for CEUS. However, the uncertainties of such measurements remain high, and quantification of microvascular flow is yet to be achieved (Sboros and Tang 2010). Microbubbles combined with ultrasound offer a number of engineering challenges, and CEUS can be further improved. In addition, the literature on the quantification of microvascular flow and volume is limited (Sboros and Tang 2010). A number of kinetics models have been proposed for destruction replenishment or bolus studies (Strouthos et al. 2010), but to our knowledge there are no *in vivo* tissue models that may aid in their development. In general, the development of CEUS has been attempting complex measurements, under elaborate experimental conditions and in challenging microvascular systems such as the myocardium and the liver. The present communication proposes the ovine ovarian corpus luteum (CL) as one such model.

Briefly, both the ovary and endometrium undergo regulated cyclic angiogenesis and vascular regression during the estrus cycle. The changes in, and regulation of, ovarian angiogenesis is an active research field (McNeilly and Fraser 1987; McNeilly et al. 1992b; Fraser and Lunn 2000; Fraser et al. 2000; Wulff et al. 2002; Souza et al. 2003, 2004) in relation to both maximizing fertility and potential treatments of reproductive pathologies that feature microvascular growth or regression (Fraser and Duncan 2009). Although the outer layer of thecal cells of the follicle has a minor vascular supply, the inner granulosa cell layer of the preovulatory follicle, separated by a basement membrane from the thecal layer, is avascular. At the time of ovulation, when the preovulatory follicle releases the oocyte, the follicle collapses and there is a marked and predictable vascularization of these steroidogenic granulosa cells. The resulting CL has a highly developed microvascular network to the extent that each steroidogenic cell is adjacent to, or very close to, an endothelial cell and the CL has a blood supply, per unit mass, eight times that of the kidney. In the absence of pregnancy, a regression of vasculature occurs and in a matter of days, the CL becomes an avascular remnant. Various hormones including the locally acting vascular endothelial growth factor (VEGF) have a role in the CL development (Fraser and Lunn 2000; Dickson et al. 2001; Wulff et al. 2001a, 2001b, 2002; Yong et al. 2003; Fraser et al. 2005). Thus, the CL provides a natural angiogenic and anti-angiogenic regulation with well-established endocrinologic monitoring, and in the sheep it can be controlled by hormone or similar treatments. It can thus be used to develop quantitative methodologies that may be of use to all microvascular imaging studies.

Contrast-enhanced ultrasound has been used to investigate ovarian pathology, with the main focus being cancer (Orden et al. 2003; Kohzuki et al. 2005; Testa et al. 2007). One study on the normal cycle of the sheep ovary, measuring contrast kinetics in the whole ovary (*i.e.*, including CL and all other vasculature), provided significant differences between the follicular and the luteal phase (Marret et al. 2006). In view of the large vascular heterogeneity of the ovary, these authors proposed selective sampling of regions for contrast analysis to improve on vascular discrimination within any lesion (Orden et al. 2003). In addition to this, as mentioned before, the CL offers a unique opportunity for a model system that is central to an investigation on the quantification of microvascular flow. To our knowledge, such an experimental *in vivo* model has not been reported previously and in the present communication, we aim to establish this *in vivo* model for the development of microvascular imaging using CEUS. Real-time imaging of the microvascular blood flow of the CL with low mechanical index (MI) was adopted. To achieve our aim, a quantification scheme for the extraction of hemodynamic-related parameters followed. The experimental uncertainty, as well as comparison with the gold standard of histology, is presented. The potential use of the CL model to simulate vascular regression is explored.

MATERIALS AND METHODS

Animal protocol

These studies were performed at the MRC Human Reproductive Sciences Unit (Edinburgh, UK), and all animal procedures were approved and conducted in accordance with the Home Office Animals (Scientific Procedures) Act 1996 of the United Kingdom. Six Scottish black-face ewes (age, 4 to 5 y; body weight, 55 to 66 kg) exhibiting normal reproductive cycles and having had at least two successful pregnancies were used in this study. The perfusion of fully functional CL was studied on days 8–12 of the estrous cycle in six sheep. In November, which is within the fertile season for the sheep, progestogen-impregnated sponges (60 mg medroxy-progesterone acetate pre-sponge; Intervet Laboratories Ltd., Cambridge, UK) were inserted in all animals. After 14 d *in situ*, sponges were withdrawn, and on day 12 of the subsequent estrous cycle, luteal regression was induced by prostaglandin F2alpha (100 μg intramuscular cloprostenol; Estrumate; Coopers Animal Health, Crewe, Cheshire, UK). Progesterone was measured in blood samples from each ewe taken on day 6 of this second estrous cycle and assayed by nonextraction radioimmunoassay (sensitivity 0.2 ng/mL; intra-assay